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# High Prevalence of Rodent-Borne *Bartonella* spp. in Urbanizing Environments in Sarawak, Malaysian Borneo

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*Abstract.* Rodents are the most prominent animal host of *Bartonella* spp., which are associated with an increasing number of human diseases worldwide. Many rodent species thrive in urban environments and live in close contact with people, which can lead to an increased human risk of infection from rodent-borne pathogens. In this study, we explored the prevalence and distribution of *Bartonella* spp. in rodents in urban, developing, and rural environments surrounding a growing city in Sarawak, Malaysian Borneo. We found that although *Bartonella* spp. infection was pervasive in most rodent species sampled, prevalence was highest in urban areas and infection was most commonly detected in the predominant indigenous rodent species sampled (*Sundamys muelleri*). Within the urban environment, parks and remnant green patches were significantly associated with the presence of both *S. muelleri* and *Bartonella* spp., indicating higher localized risk of infection for people using these environments for farming, foraging, or recreation.

The genus *Bartonella* contains a diverse group of emerging, zoonotic, gram-negative, facultative intracellular *Alphaproteobacteria* that infect a wide range of wildlife and domestic animals. Rodents appear to be the most common wildlife host of *Bartonella*, having been associated with 20/33 described species to date, many of which have also been linked to disease in people.[1,2] Transmission between animal hosts appears to be primarily through ectoparasite vectors, including fleas, mites, and ticks.[2] However, it is clear from the diversity of recently described *Bartonella* spp. associated with a widening range of mammalian hosts and potential arthropod vectors that the ecology of these bacteria is complex and not well understood.[3,4]

In people, infection with *Bartonella* often results in undifferentiated febrile illnesses that may be similar in clinical presentation to those caused by other pathogens (e.g., *Borrelia* spp.).[2,5] This suggests that the global burden of *Bartonella*-associated diseases, although significant, may be underestimated. Some groups of people (e.g., outdoor workers, immunocompromised people, and the homeless) may be particularly vulnerable to infection, indicating that human behavior and local ecology may be significant contributors to zoonotic disease risk.[2] In this study, we screened indigenous and invasive rodents found in urban, developing, and rural locations around the city of Kuching, Sarawak, for *Bartonella* spp., to begin to explore the role of local ecology in the presence and prevalence of *Bartonella* in Malaysian Borneo.

As part of a larger study on the effect of urbanization on rodent-borne diseases, we collected 316 rodents from several sites in urban, developing, and rural areas in and around the city of Kuching, Sarawak, between September 2015 and April 2016 (Supplemental Figure 1). The predominate land use type at each site was characterized by estimating the proportion of green or gray space within 10 m and 100 m radii from the trapping site and by the proportion of forest cover. Mean forest cover was estimated using QGIS v 2.14.0 and previously published forest cover and loss datasets at the Landsat pixel scale, ranked and grouped into tertiles, which were categorized as minimal, moderate, or maximal forest cover (https://earthenginepartners.appspot.com/science-2013-global-forest). Rodents were live-trapped using locally made wire-mesh traps and euthanized by over-anesthetization in isoflurane, followed by bilateral thoracotomy. Tentative species assignment, gender, breeding status, and body mass (as a proxy for age) were recorded, and tissues and ectoparasites (i.e., mites, lice, fleas, and ticks) were collected and frozen directly on dry ice. The species identity of each animal was confirmed using primers BatL5310 and R6036R, which amplify ~750 bp of the cytochrome oxidase I gene.[6] Based on the resultant sequences, rodents grouped with eight species from four genera, with most individuals falling within the *Rattus rattus* super-group ($N$ = 187) or classified as *Sundamys muelleri* ($N$ = 100) (Supplemental Table 1). Although three species of the *R. rattus* super-group were delineated by this method (i.e., *Rattus* sp. R3, *Rattus tanezumi*, and *Rattus tiomanicus*), we considered them collectively for this analysis, as distinct mitochondrial lineages of this super-group are known to hybridize when sympatric.[7,8] All animals and samples were collected with permission from the Commonwealth Scientific and Industrial Research Organization AAHL Animal Ethics Committee (#1750) and the Sarawak Forests Department (#NCCD.907.4.4(JLD.12)-131).

DNA was extracted from ~30 mg of rodent spleen homogenate using the AllPrep DNA/RNA mini Kit (Qiagen) and subjected to a nested polymerase chain reaction (PCR) targeting the *Bartonella* citrate synthase A (*glt*A) gene using a nested PCR reaction. Positive samples generated either a 767-bp product in round 1 (primers CS443f and CS1210r) or a 694-bp product in round 2 (primers CS443f and BhCS.1137n) of the PCR and were confirmed by Sanger sequencing.[9,10] The resultant sequences (GenBank accession nos. MG807665–MG807845) were trimmed for quality and length and were manually aligned with those of a representative sample of *Bartonella* spp. in Geneious version 10.2.2.[11] A maximum likelihood (ML) phylogenetic tree was constructed using the Generalized Time Reversible plus gamma model of nucleotide substitution in PhyML v3.1, with 1,000 bootstrap replications.[12] Sequences were then trimmed to include only the 327-nt region of *gltA* (positions 801–1127) commonly used for

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